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INTERACTION OF SERUM LEUKOCYTES, AND BACTERIA IN PHAGOCYTOSIS AS OBSERVED IN A CASE OF RECURRENT AND RELAPSING ERYSIPELAS.*†

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WRIGHT and his followers have maintained that the serum is the only variable element in phagocytosis *in vitro*, and that the source of the leukocytes is immaterial. Other investigators in the last three years have shown that this position is probably not tenable, and that there are variations in the phagocytic power both of the normal leukocytes and of leukocytes from cases of disease.

Rosenow[1] found that the leukocytes in pneumonia are perceptibly more actively phagocytic than normal leukocytes, a property which seems independent of the serum. They are more resistant to heat, and when heated to the thermal death point, and resuspended in serum, they are more actively chemotactic for pneumococci. Potter and Krumwiede[2] found that "just after the crisis or lysis the phagocytic power of pneumonic leukocytes increases rapidly, surpasses, and then later equals or becomes less than that of normal leukocytes."

Briscoe[3] states that the different varieties of leukocytes according to Arneth's[4] classification have distinctly different phagocytic powers, those of class III being most active, and those of class I least active. Pottenger[5] arrives at a similar conclusion from studying the leukocytes of normal persons and of cases of tuberculosis, and endeavors to explain the low resistance in certain cases of high leukocytosis by the fact that the new leukocytes may consist largely of classes I and II which have a much lower phagocytic power than the other classes so that the total phagocytic power of the blood may be much less than would seem to be indicated by the white count alone. Glynn and Cox[6] in a recent article have made careful and extended observations on the phagocytic power of leukocytes from three normal persons, from one case of mild chronic staphylococcus infection, and from a number of miscellaneous cases, infectious and otherwise. They find that the "inherent phagocytic power" of leukocytes (tested with Staph. albus and tubercle bacillus) varies fully as much as the opsonic index and probably more. In normal persons they found this "cytophagic index" to vary from 0.54 to 1.22 as compared with other normal persons.

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[1] *Jour. Infect. Dis.*, 1906, 3, p. 683.

[2] *Ibid.*, 1907, 4, p. 601.

[3] *Brit. Med. Jour.*, 1907, 2, p. 1493.

[4] *Die Neutrophilin weissen Blutkörperchen*, Jena, 1904.

[5] *Jour. Amer. Med. Assoc.*, 1909, 52, p. 1980.

[6] *Jour. Path. and Bact.*, 1909, 14, p. 90.

Ledingham[1] seems to have been the first one to point out a specific reaction between patient's leukocytes and patient's serum whereby a patient's leukocytes may be much more or less actively phagocytic in his own serum than are normal leukocytes under similar conditions, while in normal serum this difference may not appear. As a result of this relation the opsonic index will be higher or lower when determined with the patient's leukocytes than when normal leukocytes are used. He found in certain cases of myelogenous leukemia that the serum may have quite a normal opsonic content when tested with normal leukocytes, but a markedly subnormal one if tested with the patient's own leukocytes. On the other hand, in a case of chronic lymphatic leukemia, the polymorphonuclear leukocytes were much more actively phagocytic than normal leukocytes. It was also found that the leukocytes of the leukocytosis induced by cinnamate of sodium had much less phagocytic activity than normal leukocytes. Shattock and Dudgeon[2] found that lekocytes in infectious diseases may show considerably more or less phagocytic activity toward melanin and colon bacillus than normal leukocytes, and this relation may hold true when suspended either in their own serum or in a foreign serum (normal or from a case of infectious disease). They conclude that "immune or active cells, as compared with normal cells, altho they usually do more work in both immune and normal serum, do more work in the immune than in the normal serum." They consider this to be a peculiarity inherent in the cell itself, and not due to any stimulation by the serum. Bayly[3] tested the opsonic index (bacterium not mentioned) in 44 parturient women, and found that altho the index when determined with normal leukocytes was within normal limits in nearly all cases, yet when determined with the patient's own leukocytes, the index was lower in 38 cases; in four cases it was higher. He did not determine whether this was due to a decreased activity of the patient's leukocytes (as compared with normal leukocytes) when in their own serum, or to an increased activity when in a foreign serum. Parvu[4] studied phagocytosis of typhoid bacillus and staphylococcus in a case of myelogenous leukemia. His observations correspond with those of Ledingham, namely, that the opsonic index may be lower when determined with the patient's own leukocytes than when normal leukocytes are used. He also observed that many polymorphonuclear leukocytes lose the power of phagocytosis, and that this function is taken over to a considerable extent by the myelocytes. In his calculations he considered only the polymorphonuclear leukocytes as phagocytes. LePlay[5] studied the opsonic index in two cases of gonococcus infection, one of them complicated with typhoid fever. He found the index determined with the patient's leukocytes often lower than when determined with normal leukocytes. He says: "The index determined with the leukocytes of the patient and the serum of the patient, as compared with the serum and leukocytes of normal persons, is the one that falls most and rises most."

None of the authors quoted thus far has considered, or at least none has demonstrated, that this reaction between patient's leukocytes and patient's serum may be specific for the infecting bacterium in the particular case. The non-specificity for bacteria of variations in the phagocytic activity of patient's leukocytes is shown particularly well in the work of Shattock and Dudgeon in which they used melanin in a large proportion of their experiments. And it is shown also, of course, in these cases of non-infectious disease studied by several authors. The fact that variation in the phagocytic activity of leukocytes in the presence of homologous serum in some cases may be specific for the infecting bacterium seems to have been first pointed out by Rosenow.[1] Rosenow found, in a case of pneumococcus endocarditis, that, when using the infecting strain, the opsonic index was lower when obtained with the patient's leukocytes than when normal leukocytes were used. But when a strain of pneumococcus obtained from a case of pneumonia was used, the index was slightly higher with the patient's own leukocytes than with normal leukocytes. In other cases of pneumococcus endocarditis, he failed to find such a specific relation. In one case he found that altho patient's leukocytes readily ingested bacteria either in their own or in normal serum, yet patient's leukocytes in patient's serum had no power to destroy pneumococci; patient's leukocytes in normal serum, however, or normal leukocytes in patient's serum readily destroyed the bacteria. This reaction was not specific for the infecting strain. But in a case of pseudodiphtheric cystitis, Rosenow[2] found the opsonic curve determined with the patient's leukocytes to be quite different from that obtained with normal leukocytes and to show a more favorable response to bacterial inoculations. The curve of the phagocytic power of the leukocytes also showed a favorable response on the part of the leukocytes to the inoculations.

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[1] *Aberdeen University Studies*, 1906, 21, p. 321.

[2] *Proc. Royal Soc.*, Lond., 1907, 80, B, p. 165.

[3] *Lancet*, 1908, p. 1073.

[4] *Comp. rend. de la Soc. Biol.*, 1908, 65, p. 480.

[5] *Ibid.*, 1909, 66, p. 930.

Because of such variations in the leukocytes as well as in the serum, some observers have recommended that an index be obtained by using the whole blood from the patient, whole normal blood being the standard for comparison. This has been called the "hemophagocytic" index, the "opsono-cytophagic" index, and by other names. This is the method originally used by Leishman, and almost lost sight of after the introduction by Wright and Douglas of the opsonic index. The advantage of this index is that it takes into consideration all sorts of variations in the leukocytes as well as in the serum, but the presence of a leukocytosis would be expected to lower this index instead of raising it, for the larger number of leukocytes in the same volume of the mixture probably would ingest a smaller average number of bacteria. Glynn and Cox (*loc. cit.*) lay great stress on this index, which they obtain by suspending patient's washed leukocytes in the homologous serum and comparing them with normal leukocytes in normal serum. They assert that this index can be accurately calculated from separate determinations of the opsonic index and of the phagocytic power of the leukocytes (the "cytophagic" index). In my case this index could not have been so calculated.

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[1] *Jour. Infect. Dis.*, 1909, 6, p. 245.

[2] *Ibid*, 1909, 6, p. 296.

My own work was carried out principally in connection with the following case of recurrent and complicated erysipelas.

A laborer, single, 32 years of age, had a first attack of erysipelas, facial type, in April, 1908; the second attack in October, 1908, was also facial, later migrating down the back. On leaving the hospital the left ear suddenly became painful; after a few hours the membrane ruptured, and a profuse discharge of pus occurred, which continued until May, 1909, when, following a recurrence, the discharge disappeared almost completely, and thenceforth remained but slight. The third attack was in December, 1908, beginning in the left ear and extending down the back. The patient remained in the hospital constantly from this attack until his death (May 20, 1909). During the next four weeks after this attack three relapses occurred. It was at this time that the patient came under observation, and I am indebted to Dr. Weaver and to Dr. Friedberg for the opportunity of studying the case in their wards at the Cook County Hospital. Following this last attack streptococcus inoculations were begun. Subsequently the patient had four recurrences and one relapse, making a total of six recurrences and four relapses. Two of these attacks began in the discharging ear, and all of the others either on the cheek or the nose. With the exception of the first, every attack assumed the migrating type. There was no history of nasal catarrh, or of sinus infection, tho the patient stated that during each attack of erysipelas there had been a moderate nasal discharge, and when first seen there was slight tenderness over both supramaxillary sinuses. The nasal mucus had been slightly blood-tinged for several years.

The pus from the ear contained a nearly pure culture of hemolyzing streptococci. Cultures were made on blood agar, killed with 25 per cent solution of galactose,[1] and on January 20, 50,000,000 cocci were injected. At this time the opsonic index, determined in the ordinary way, was 0.36, and the leukocyte count 11,950. The next day the index rose nearly to normal, and remained there until the sixth day, when another injection of 50,000,000 was given; the index now rose abruptly to 2.5, and then fell abruptly to 1.3, thence declining slowly to normal. A subsequent injection of 50,000,000 cocci produced no effect upon the index. Three days later 150,000,000 cocci were injected. No immediate effect was produced on the index, but the next day the patient showed indications of acute endocarditis; and the day after this he developed a multiple arthritis which lasted several days. Four days after this inoculation he received another inoculation of 400,000,000 cocci, and the index rose to 1.8. Two days later his erysipelas recurred. Eight days later he had a relapse. A blood culture at this time was sterile. Two weeks later the mastoid process was opened, and a subdural abscess containing about an ounce of pus was drained. The general condition of the patient was apparently not improved by the operation.

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[1] Weaver and Tunnicliff, *Jour. Infect. Dis.*, 1908, 5, p. 589.

Because of the fact that the phagocytic power of the patient's blood was considerably increased by the addition of a small amount of normal human serum, and was increased even more by normal horse serum (see Table 5), the patient was injected on March 14, 1909, with 10 c.c. of fresh normal horse serum.[1] This was five days after the mastoid operation, from the immediate effects of which he had quite recovered. He was feeling a little weak, but just about the same as before the operation. The injection was given about noon. In a short time he began to feel much better; that evening he ate a hearty supper, and sat up for a few hours—neither of which he had previously been able to do since his operation; that night he slept better than he had for a long time. The next morning he still declared that he felt better, but his erysipelas had recurred. The attack was a slight one, and he recovered completely from it in about five days. In view of the remarkable tho transitory improvement in the general condition, the recurrence was looked upon at that time as possibly a coincidence.

Two weeks later 500,000,000 streptococci were injected. There was no local reaction, but the next day there was moderately severe pain in the unaffected ear. A couple of days later there was considerable stiffness, and pain on motion in the muscles of the back of the neck. There was slight tenderness over the linea nuchae, but none over the mastoid. In the course of a week or so these disagreeable symptoms disappeared, and he seemed to be somewhat better. This was interpreted as being a positive reaction following a somewhat severe and prolonged negative phase. It is questionable if there was any net gain. About five weeks after the first injection of horse serum when the patient was in fair condition, 8 c.c. of fresh normal horse serum were injected into the right arm. The next day there was a rather severe local serum reaction and the temperature had risen somewhat. During the next four days the general condition became worse; the patient was weaker, the temperature reached 101° F., or 102° F. every day, being remittent in type, the pulse was accelerated, and the discharge from the mastoid became profuse and bloody. The cutaneous reaction extended, but was limited to the arm. The whole affair was probably a severe form of serum disease, tho it presented many points of similarity to a general septicemia. Finally, on the sixth day after the injection, there was a recurrence of the erysipelas, which promptly migrated down the entire back. This attack lasted six days, and then the mastoid discharge suddenly stopped, and the wound closed up almost completely. After that there was a slight mucous discharge but no pus. On the fourth day of the attack, the patient developed an acute pleurisy. The chest was twice aspirated and both times a thick fluid containing flocculi of fibrin was obtained. Unfortunately no smears or cultures were made.

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[1] Therapeutic injections of normal horse serum have been tried and recommended by a number of observers, altho the rationale of this method of treatment is not clear. It is known, however, that the effects of a foreign serum may be quite different *in vivo* from what might be expected from experiments *in vitro*.

Cole and Smirnow (*Bull. Johns Hopkins Hosp.*, 1908, 19, p. 249) have shown that a property similar to that ascribed by Bail to his so-called "aggressins" may be present in normal serum. They noted that pigeons normally possess a surprising degree of resistance to B. pneumoniae. But pigeon serum, when injected into susceptible animals with a dose of living bacteria, instead of protecting them. rendered them more susceptible than controls. Rabbit serum produced a similar effect on mice.

It seemed clear from these two experiences that the horse serum was responsible for the recurrence in both instances. The delay in the second recurrence may have been due to some immunity having been acquired which postponed the attack for a few days, and it may be that the severe serum disease had the effect of reducing the patient's resistance and allowing the latent infection to assert itself.

Following this attack of erysipelas, altho the condition of the ear improved so remarkably, the patient's general condition became worse and in about two weeks he had another recurrence. He recovered completely from the erysipelas in about six days, but the fever remained, being intermittent in type, and reaching 103°–104° F. every day; four days later (May 20, 1909) he died.

An interesting phenomenon was observed during each of the last two attacks. About 24 hours before the appearance of the typical erysipelatous eruption, a patch of dull red was observed upon the nose. This was not the seat of any subjective sensations and was not in the least elevated or edematous. It did not have the tense, shiny appearance of a typical patch of erysipelas, nor the sharp contour. It was merely an indefinite erythematous patch. At the same time, or 24 hours earlier, the skin at the back of the neck and between the shoulders presented an edematous appearance. There was no distinct patch, there was no redness, there were no symptoms, and the edema itself was very much softer than that of an erysipelatous patch, pitting quite readily. The typical erysipelatous eruption occurred almost simultaneously in these two areas. These appearances could hardly be interpreted as a typical form of the eruption, for when the eruption came it was perfectly typical; perhaps they represent a true prodromal eruption.

Following the early inoculations a mild local reaction was usually obtained. It was never troublesome, and never lasted longer than two days. Later this reaction failed to appear. The leukocyte count showed great fluctuations (5,000 to 23,000) which did not seem to be related to the clinical condition, to the opsonic index, or to the inoculations. The percentage of polymorphonuclears remained close to 90 per cent, and late in the disease the stained specimen showed considerable variation in the size and staining properties of the leukocytes. No unusual types were seen. The hemoglobin early was 90 per cent; March 20 it was 57 per cent, and on May 14 it was 67 per cent (Sahli).

On February 9, the day before the third recurrence, and after the patient had received five inoculations, it was observed that the index determined with the patient's leukocytes was much lower than when determined with normal leukocytes, that is to say, the patient's leukocytes, while acting as well as normal leukocytes in normal serum, showed but little phagocytosis in the homologous serum (see Table 1). This phenomenon was observed repeatedly. Table 1 shows the phagocytic indices obtained in the various combinations made by using the leukocytes and serum from a normal person, from this case—designated in the table as M—and from a case of convalescent erysipelas. The suspensions of leukocytes in this and the succeeding experiments were of practically equal density. The following method of standardization was used: Suspensions of leukocytes were first made from the "cream" of the washed and centrifuged specimens of the different bloods to be compared, in the ordinary way. Then by means of a high power lens and the ruled stage, the number of polymorphonuclear leukocytes in a given volume of each suspension was determined. The stronger suspensions were then diluted until they were of the same density as the weakest.

TABLE 1.

Phagocytic indices obtained by using serum and leukocytes from one normal person and from two cases of erysipelas, the leukocytic suspensions being of equal density.

LEUKOCYTES	SERUM		
	Normal	Convalescent Erysipelas	Case M
Normal	3·13	3·20	5·5 (index 1.76)
Convalescent erysipelas	3·6	3·97	5·72 (" 1.59)
Case M	3·56	4·98	0.76 (" 0.21)

This table shows that tho the index of the patient's serum (Case M) when obtained with normal leukocytes or with the other patient's leukocytes is high, 1.76 and 1.59, the index obtained with this patient's own leukocytes is very low, 0.21. The fact that the patient's leukocytes acting in other serums may give even a greater degree of phagocytosis than normal leukocytes, shows clearly that the fault lies not with the leukocytes but with the serum. This may be due to one of two things or to both: either some element necessary to phagocytosis, and present in normal serum, is largely absent in this patient's serum, or else there is an inhibitory substance present which is specific for the patient's leukocytes. The evidence on this point is not conclusive, but the next two tables show some figures which tend to support the first view.

TABLE 2.

Phagocytic and opsonic indices obtained by using normal and patient's serum, and suspensions of normal and of patient's leukocytes, and of patient's leukocytes which have been "soaked" in normal serum for one hour in the incubator, and then washed.

LEUKOCYTES	PHAGOCYTIC INDEX		OPSONIC INDEX
	Normal Serum	Patient's Serum	
Normal	10.94	4.92	0.45
Patient's	16.04	1.63	0.10
Patient's treated with normal serum	24.88	10.43	0.42

TABLE 3.

Showing the increase in phagocytic index obtained by adding a small amount of normal serum to patient's serum, and comparing this index with that obtained with normal leukocytes and normal serum.

Leukocytes	Serum	Phagocytic Index
Patient's	Patient's	1.63
"	" +1/20 vol. normal serum	11.72
"	NaCl + " " " "	1.78
Normal	N.	10.94

NOTE.—The phagocytic mixtures in these experiments consisted usually of 1 volume of serum, 1 volume of suspension of leukocytes, and 1 volume of suspension of bacteria. In this and succeeding tables where the term "1/20 vol." is used, it means 1 volume of serum which has been diluted in the ratio of 1:19 with normal salt solution.

In Table 2 the index of the patient's serum when determined with normal leukocytes is 0.45 and when determined with the patient's own leukocytes is only 0.10. But when the patient's leukocytes are first "soaked" in normal serum for one hour in the incubator, and washed before using, the index is practically the same as when normal leukocytes are used. In Table 3 it is seen that when a small volume of normal serum is added to the patient's serum, the opsonic power of the patient's serum (with respect to patient's leukocytes) is enormously increased. This would seem to indicate that there is some substance necessary to phagocytosis present in normal serum, which is largely or wholly lacking in this patient's serum and that it need be present only in small amount because the opsonic power of a given volume of the patient's serum may be considerably increased by the addition of so much of this substance as is contained in 1/20 of that volume of normal serum. Moreover, this substance may be taken up by the patient's leukocytes when exposed to normal serum and so bound as to resist repeated washing. Whether this substance is taken up mechanically by the leukocytes, or whether there is a firmer union, cannot be stated definitely.

It would be natural to suppose that this substance which is lacking is complement, or at least a complement-like body, for the patient's fresh serum acts much like the heated serum of those individuals whose sera show the phenomenon of reactivability, but this is not necessarily true, for this patient's serum is not reactivable, as the following table shows:

TABLE 4.

Sera which activate the patient's fresh serum will not reactivate the heated serum. Patient's leukocytes only are used.

Serum	Phagocytic Index
Patient's	0.94
" +1/20 vol. normal serum	5.54
" +1/20 " serum "S"	4.36
" (heated)	0.50
" " +1/20 vol. patient's serum	0.33
" " +1/20 " normal "	0.30
" " +1/20 " serum "S"	0.14
Normal serum diluted 1/20	0.20
Serum "S" diluted 1/20	0.35

These figures show that the patient's heated serum is not reactivated by a small volume of patient's fresh serum nor by a small volume of either of two other sera, tho these same sera are able to activate the unheated serum.

This does not mean definitely that the activating substance is not complement, for there is still the possibility that lack of reactivability in a given serum is due, not to the lack of thermostable substance, but to an inhibition produced by the heating. In fact the figures of Table 4 might almost be urged in support of that view.

The effect of heat and age on the power of normal serum to activate this patient's serum is shown in Table 5.

TABLE 5.

Old or heated serum possesses less reactivating power than fresh serum.

Leukocytes	Serum	Phagocytic Index
Patient's	Patient's	0.94
"	" +1/20 vol. normal serum	5.54
"	" +1/20 " " (heated)	2.12
"	" +1/20 " " " (old)	1.72
"	" +1/20 " " horse serum	7.60
"	" +1/20 " " " " (old)	1.48
"	$\frac{1}{20}$ vol. normal serum	0.20
"	$\frac{1}{20}$ " horse "	0.22

The "old" human serum used here was six days old, and the "old" horse serum four months old; both had been kept in the ice-box.

These figures show that old serum and heated serum (60° for 30 m.) lose much but not all of their power of activation. Incidentally it is seen that normal horse serum has a greater activating effect than normal human serum and hence its injection into the patient.

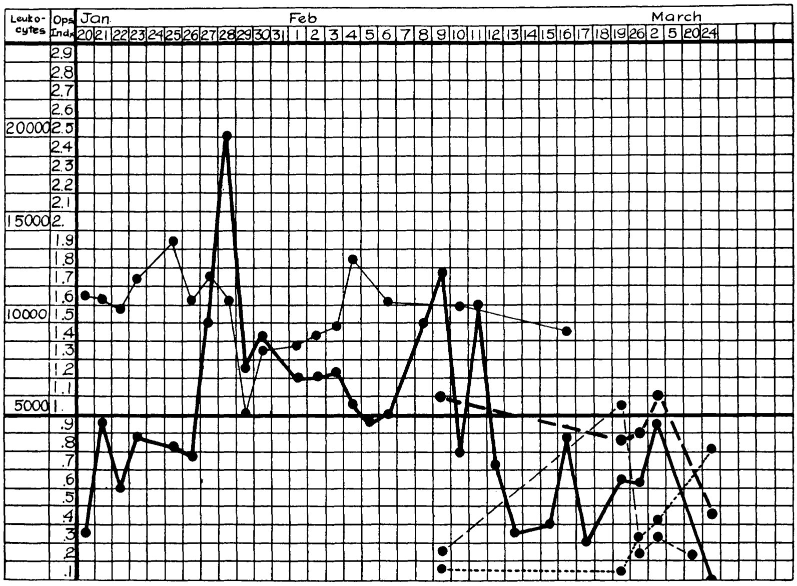

CHART 1.—Solid heavy line=opsonic index obtained in the usual way with normal leukocytes.

Fine broken line=opsonic index obtained in the usual way with the patient's leukocytes.

Heavy broken line=phagocytic power of the patient's leukocytes in normal serum as compared with the phagocytic power of normal leukocytes in normal serum.

Dotted line=phagocytic power of the patient's leukocytes in the patient's serum as compared with the phagocytic power of normal leukocytes in the patient's serum.

Solid fine line=leukocytic curve.

It has been suggested that possibly the low opsonic power of this patient's serum with respect to his own leukocytes might be due to an inhibitory substance which is neutralized by normal serum and which either does not affect normal leukocytes or is neutralized by them. I am able to present very little direct evidence in favor of such a view, but everything that has been said so far in favor of the opposite theory might be explained equally well by this one. Results have been obtained a few times which suggest such an inhibitory action, but the differences obtained have never been marked. On the other hand, normal serum appeared at times to exert an inhibitory action on the patient's serum. In another case (S) using patient's leukocytes and patient's serum, the phagocytic index was 1.91; when 1/20 volume of normal serum was added the index was reduced to 0.62.

Results have been obtained which indicate variations in the phagocytic power of the leukocytes, independent of changes in the serum. In many of the experiments the patient's leukocytes were practically normal when in normal serum, but sometimes the leukocytes were more active than normal, and on two occasions they were less active than normal leukocytes when in normal serum (once slightly so, and once markedly so).

In Table 1 the patient's leukocytes (Case M) are seen to be more active than normal leukocytes when in another patient's serum tho that serum has a normal index, yet in the homologous serum they show very little phagocytosis. In Table 2 it is seen that the patient's leukocytes when in normal serum are considerably more active than normal leukocytes, altho the index of the patient's serum is low (0.45). In Table 6 are shown results obtained by using the infecting and one other streptococcus in various combinations of serum and leukocytes.

TABLE 6.

THE APPARENT SPECIFIC RELATIONSHIP BETWEEN LEUKOCYTES, SERUM, AND STREPTOCOCCI IN CASE M.

LEUKOCYTES	PHAGOCYTIC INDEX		OPSONIC INDEX	
	Normal Serum	Patient's Serum		
Normal........	4.32	1.25	0.30	Infecting streptococcus
Patient's.......	2.52	1.85	0.73	
Normal........	9.73	6.42	0.66	Laboratory streptococcus
Patient's.......	8.26	8.82	1.06	

These figures were obtained on the day that the patient was recovering from a relapse, and so it might be expected that the defensive powers would be at their height. In both instances the opsonic index of the patient's serum obtained by using the patient's leukocytes is higher than that obtained by using normal leukocytes; in both instances the index is lower with the infecting streptococcus than with another strain. In the case of the infecting streptococcus, the patient's own leukocytes in normal serum are less active than normal leukocytes, altho in their own serum they are fully as active as, or possibly more active than, normal leukocytes. In the case of the stock streptococcus the patient's leukocytes in normal serum are practically normal, and when in their own serum are even more active than normal leukocytes. A comparison of these figures suggests an appreciable degree of specificity in the triple relations of serum, leukocytes, and streptococci. From these figures it is difficult to say wherein this specificity resides, but it seems almost certainly to involve both serum and leukocytes; for it is shown by the leukocytes in normal serum and by the serum when using normal leukocytes. These figures assume all the more significance in view of the general uniformity in the behavior of any given serum toward various strains of streptoccoci grown under similar conditions. In the following experiment the results show that these leukocytes may behave differently toward different streptococci in the same normal serum:

TABLE 7.

LEUKOCYTES	PHAGOCYTIC INDEX		OPSONIC INDEX	
	Normal Serum	Patient's Serum		
Normal........	6.0	6.16	1.00	Infecting streptococcus
Patient's.......	4.0	1.43	0.36	
Normal........	8.0	4.88	0.61	Laboratory streptococcus
Patient's.......	7.22	1.56	0.51	

On this occasion the index was higher for the infecting strain and was lower when obtained with the patient's leukocytes (the exact reverse of the conditions in the previous experiment), but one relation remains the same, viz., while the patient's leukocytes in normal serum are practically normal for the stock streptococcus, they are somewhat less active than normal for the infecting strain. The difference is slight but suggestive.

The deficiency of the patient's leukocytes is more clearly shown in this experiment in which the infecting streptococcus was used. Here, altho the patient's leukocytes are practically normal in his own serum, they are much less active than normal in normal serum, and consequently the index is slightly higher with patient's leukocytes than with normal leukocytes:

TABLE 8.

LEUKOCYTES	PHAGOCYTIC INDEX		OPSONIC INDEX
	Normal Serum	Patient's Serum	
Normal........	12.20	1.16	0.095
Patient's......	6.80	0.94	0.14

In an attempt to throw some light on the apparent discrepancy between the action of horse serum *in vivo* and *in vitro*, the phagocytic power of serum and leukocytes was tested with respect to the second generation of a recently isolated strain of a hemolytic streptococcus obtained from the patient's ear, grown on blood agar, and kept in the ice-box, and with respect to a strain also obtained from the patient, and grown on plain agar in the laboratory for three months.

TABLE 9.

THE DIFFERENCE IN THE OPSONIC INDEX FOR DIFFERENT STRAINS OF STREPTOCOCCI FROM THE PATIENT.

Leukocytes	Serum	Phagocytic Index		
Normal	Normal	6.16		
Patient	Patient	3.87	0.62	Hemophagocytic index for long-cultivated strain
"	" +1/20 vol. normal serum	6.48	0.95	
Normal	Normal	6.88		
"	Patient	12.75	1.90	Opsonic index for recently isolated strain
Patient	Normal	6.01		
"	Patient	12.18	2.03	
"	" +1/20 vol. normal serum	6.81	1.13	

The interesting fact is here shown that while the index is low for the strain that has been cultivated for three months (0.62), it is high for the strain recently isolated (1.9). This index is practically the same whether determined with the patient's leukocytes or with normal leukocytes. Furthermore, the addition of a small amount of normal serum to the patient's serum raised the index for the former (from 0.62 to 0.95), while it lowers the index for the latter strain (from 2.03 to 1.13). This might be urged to be an adequate explanation for the bad results obtained by injection of the patient with fresh horse serum.

Several other cases of relapsing and of migrating erysipelas were tested for the possible presence of peculiar relations between serum and leukocytes similar to those found in this case. The results of this investigation are reported elsewhere.[1]

SUMMARY.

The principal observations in this case may be briefly summarized as follows:

Inoculations of the infecting streptococci killed by galactose solution, in doses of 50,000,000 to 500,000,000, were of doubtful benefit. Early in the course of the disease the opsonic index was raised, but no considerable improvement in the local or general condition can be ascribed to the inoculations. The results of the phagocytic experiments indicate that there may be a certain degree of specificity in the relations of serum leukocytes and microbes (in this case streptococci) to one another. This specificity for the infecting streptococci was shown in this case by the serum independently of the leukocytes and by the leukocytes independently of the serum, but it was most marked when leukocytes were suspended in their own serum. Moreover, there was a specific relation between the patient's serum and leukocytes which was different for different strains of streptococci. At times the opsonic index obtained with the patient's leukocytes was much lower than when obtained with normal leukocytes. This was owing either to the lack in the serum of some element necessary to phagocytosis which is present in normal serum (and in normal leukocytes), or to some inhibiting substance specific for the patient's leukocytes and neutralized by normal serum. When a small amount of normal serum (1/20 volume) was added to the patient's serum, or if the patient's leukocytes were first "soaked" in normal serum and then washed, the index determined with the patient's leukocytes was practically the same as when determined with normal leukocytes. When the serum was heated it lost much of its opsonic power and could not be reactivated by sera that would activate it in the unheated state. The fact that the patient's leukocytes gave a lower opsonic index than normal leukocytes has sometimes been due to an increased activity of the patient's leukocytes in a foreign serum while normal in their own. Sometimes a higher index was obtained with the patient's leukocytes than with normal leukocytes and this might be explained by a greater activity of the leukocytes in their own serum, a decreased activity in normal serum, or by both of these factors. At times the patient's leukocytes were less active than normal leukocytes in normal serum while in their own serum the difference was less marked. The serum, then, showed considerable variations in opsonic power either above or below normal independent of the conditions of the leukocytes, and the leukocytes may show variations in phagocytic power dependent however, to some extent at least, on the condition of the serum. When the patient's leukocytes were normal in phagocytic power in normal serum the patient's serum was either above or below normal in opsonic power when tested with normal leukocytes. When the patient's leukocytes were below normal the serum was either normal or below—never above. When leukocytes were above normal (one instance) the serum was also above normal. When the leukocytes tested in their own serum were normal (one instance) the serum tested with normal leukocytes was above normal. When the leukocytes in their own serum were above normal the serum was below normal. When the leukocytes in their own serum were below normal the serum was either above or below normal. The changes in the serum appeared earlier and were more persistent, more irregular, and more marked than were the changes in the leukocytes. The opsonic index was sometimes higher, sometimes lower for the infecting than for the laboratory streptococcus; and for a streptococcus strain recently isolated from the patient the index was found to be much higher than for a strain also obtained from the patient but cultivated for a considerable length of time. In connection with these observations it may be pointed out that the opsonic index as ordinarily determined in reality only measures the amount of opsonin present with respect to a given bacterium and with respect to normal leukocytes; in view of the observations here recorded the opsonic index consequently cannot be regarded as a reliable indication of the phagocytic power of the patient's blood. The general effect of the opsonic variations here described is to limit the significance of the opsonic index. It is noteworthy that so far specific variations in the behavior of serum and leukocytes with respect to the infecting bacterium have been observed only in cases of chronic infection, and it may be that it is only in long-standing infections that such special adaptations on the part of both microbes and host have time to develop. Finally it should be noted that injections of fresh normal horse serum seem to have a bad effect in this case. Possibly this was owing to the fact that while fresh horse serum increased greatly the opsonic power of the patient's serum for a streptococcal strain long cultivatd outside of the body, it seemed to lower the opsonic power for a recently isolated strain. Naturally it would be highly desirable to study the action of the injection of human serum in similar cases.

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[1] *Trans. Chi. Path. Soc.*, 1909, 8.

CONCLUSIONS.

The inoculation treatment was without benefit in a case of long-standing recurrent and relapsing erysipelas.

There may be an appreciable degree of specificity in the relation of serum, leukocytes, and bacteria, to each other. The serum may show specific peculiarities with respect to both leukocytes and bacteria, and the leukocytes may show specific peculiarities for serum and bacteria.

The phagocytic power of leukocytes may differ when in their own serum and when in normal serum. Variations in the opsonic power of the serum may occur independently of variations of the phagocytic power of the leukocytes but such variation on the part of the leukocytes, however, is not entirely independent of the variations of the serum. The phagocytic power of the leukocytes may be above or below normal in their own or in a foreign serum. The changes in the serum may appear earlier and be more persistent and more marked than the changes in the leukocytes.

The low phagocytic power of leukocytes in their own serum may depend either upon the lack of some element necessary to phagocytosis or to some inhibiting substance specific for the leukocytes in question and neutralized by normal serum.

The addition of a small amount of normal human or horse serum may increase the opsonic power of the blood; this activation was in this case quite different from the reactivation of heated serum. Injections of fresh horse serum may seem to have unfavorable results, possibly because it interferes with the phagocytosis of the infecting organism.

The opsonic index may be higher for a freshly isolated strain of the infecting streptococcus than for a long-cultivated strain.